REMARKS/ARGUMENTS

I. Status of the claims

With entry of this Amendment, claims 1 and 15 are amended, claims 18-32 are canceled and claims 33-34 are added. Claims 1-17 and 33-34 are pending with entry of the Amendment.

II. Support for the amendments

Support for the amendments can be found in the specification, original claims and the figures. The amendment to claim 1 merely incorporates language that was previously relied upon from antecedent basis in the preamble. The amendment to claim 15 and new claim 33 find support in original claim 15, Example 1, and Figures 2-3. Claim 34 finds support in original claim 15. No new matter is added.

III. Anticipation rejection

The only rejection is an anticipation rejection of claims 1-17 in view of the Kong reference. The Examiner has argued that page 806, column 2 of the Kong reference anticipates claim 1. *See*, Final Office Action, page 2. Specifically, the Examiner has argued that the target DNA in the Kong references represents the "single-stranded polynucleotide" recited in claim 1 because the target sequence becomes single-stranded during the denaturation PCR step in Kong. *See, id.* The Examiner further argued that the target sequence in Kong comprises the forward primer sequences described in Kong (e.g., in Table 2 of Kong), and thus comprised "the sequences of the primers, subsequences of the primers at least 5 nucleotides long, or complements thereof." *See, id.*

In response, Applicants argued, *inter alia*, that claim 1 required that the single-stranded polynucleotide comprise sequences, subsequences or complements thereof of "the primers" wherein "the primers" had antecedent basis and therefore referred to the "primers sufficient to amplify at least two target sequences" as recited in the preamble of claim 1. *See*, Response filed December 1, 2006, page 8. Because "primers sufficient to amplify at least two

target sequences" require at least three primers, and typically four primers to amplify two different targets, the response argued:

> Thus, claim 1 requires that the "single-stranded polynucleotide" comprise at least three and likely at least four primers when PCR is used to amplify two targets, wherein a first set of two primers are used to amplify a first target and a second set of two primers is used to amplify the second target. (Response filed December 1, 2006, page 8)

Because Kong did not teach or suggest one polynucleotide that comprised at least three or four primer sequences, subsequences or complements thereof, it was argued that Kong could not anticipate claim 1.

In response to these arguments, the Examiner argued Kong teaches amplification of 8 different targets using 8 different primer pairs, thereby meeting the limitation of "at least two target sequences." *See*, Final Office Action, page 6. With respect to the "single-stranded polynucleotide", the Examiner argued that (1) the denatured targets in Kong are single-stranded (Final Office Action, page 6), (2) that because Kong teaches 8 targets and primer pairs, Kong teaches "at least two targets" and apparently the "single-stranded polynucleotide sequence comprising the sequences of the primers, subsequences of the primers at least five nucleotides long, or complements of the sequences of the primers" recited in the claim (Final Office Action, page 7). On this last point, the Examiner emphasized that the "claims do not require one polynucleotide sequence comprise four primer sequences." *See*, Final Office Action, page 7.

Applicants respectfully traverse the rejection.

As an initial matter, it does not appear from the record that the Examiner has questioned whether the methods described in the specification or examples are novel. Rather, it appears that the rejection has been a function of the claim language and whether the claim language reads on standard multiplex PCR methods. Thus, it is helpful to provide a detailed analysis of the claim language.

Amended claim 1 reads as follows:

> 1. A method of testing the integrity of primers in a multiplex amplification reaction, the amplification

reaction comprising **primers sufficient to amplify at least two different target sequences**, the method comprising,

providing in a mixture the primers sufficient to amplify at least two different target sequences and **a single-stranded polynucleotide sequence comprising the sequences of each of the primers sufficient to amplify at least two different target sequences**, subsequences of the primers at least five nucleotides long, or complements of the sequences of the primers;

amplifying the polynucleotide sequence; and

detecting the presence or absence of the amplified polynucleotide, thereby testing the integrity of the primers in the amplification reaction.

For convenience, the newly added language is underlined, and language particularly relevant for the novelty over the Kong reference is bolded. It is Applicants position that even though it appears that Kong may describe multiplex PCR reactions, the reactions do not involve "a single-stranded polynucleotide sequence comprising the sequences of each of the primers sufficient to amplify at least two different target sequences, subsequences of the primers at least five nucleotides long, or complements of the sequences of the primers." It is Applicants position that the amendments have not changed the scope of the claim, but help to clarify that the single-stranded polynucleotide has **each** of the primers, subsequences thereof, or complements thereof, wherein "**each**" necessarily includes the primers for **each** of the "at least two targets" as recited in claim 1.

An example of this is illustrated in Figures 2-3, where the single-stranded polynucleotide has forward and reverse primer sequences for Target 1, and subsequences for forward and reverse primers for Target 2. Thus, one single-stranded polynucleotide in these examples have sequences or subsequences for four primers. It is acknowledged that claim 1 does not recite "four primers" and that the claim can encompass only three primers. However, in any

case, the single-stranded polynucleotide comprises each of the primers that are used to amplify two targets.

Kong describes development of multiple primer pairs, wherein each primer pair is specific for a ribosomal RNA sequence from a different *Aeromonas* species. Thus, for example, Table 2 provides 8 primer pairs, *each* pair for amplification of a target in a different species. It is not clear to Applicants that Kong actually ever performs multiplex PCR (and if so, what primers or targets were used). Instead, as explained in the legend for Figure 2 (Kong, p. 807), PCR amplifications were performed in which DNA from a specific *Aeromonas* species was amplified with the corresponding primers as listed in Table 2. The result was a single PCR product in which the amplicon presumably comprises the two primer sequences, or complements thereof, used in the reaction. While Figure 2 as a whole describes amplification of different species, each lane of the figure only represents one species and one set of primers. Thus Figure 2 does not describe *any* reactions in which "primers sufficient to amplify at least two different target sequences" are used and thus cannot describe a single-stranded polynucleotide having each primer sequence, subsequence or complement for two targets. Thus, the experiments leading to Figure 2 of Kong do not anticipate claim 1 at least because there is no use of set sets of primers.

The Examiner appears to argue that because Kong describes 8 sets of primers and refers to "multiplex" PCR in the methods section that Kong teaches to combine the 8 sets of primers. Assuming, *arguendo* that this is true, Kong still does not teach the limitations of claim 1. As discussed above, each set of primers in Table 2 are specific for a particular *Aeromonas* species. Thus, even if, as an example, two primer sets from Kong's Table 2 were used, it appears that only one amplicon would result, and that amplicon would be the result of amplification of one primer set, and not the other. Put a different way, since each primer set is specific for one species, only one amplification would result and the product would not comprise sequences, subsequences or complements from both primer sets. Thus, there is nothing in Kong that teaches or suggests "a single-stranded polynucleotide sequence comprising the sequences of each of the primers sufficient to amplify at least two different target sequences, subsequences of the primers at least five nucleotides long, or complements of the sequences of the primers." Accordingly, claim 1 is not anticipated by the Kong reference.

Since Kong does not describe the limitations of claim 1, Kong cannot teach the limitations of the dependent claims. Nevertheless, for clarity, several dependent claims are discussed below.

Claim 2 recites that the "target sequences are less than 50% identical to each other." Kong's primers in Table 2 amplify highly conserved sequences as shown in the Figure 1 alignment. The target sequences are *not* less than 50% identical. The Examiner argued that Kong observed no significant homology between *Vibrio cholerae*, *S. enterica*, *E. coli*, and *Aeromonas*. *See*, Final Office Action, page 3. However, this is irrelevant as there does not appear to be any amplification reaction in Kong that was used to amplify *Vibrio cholerae*, *S. enterica*, or *E. coli* sequences. Sequences from these genera were simply compared in a computer database. *See*, Kong, page 805. Thus, in addition to the differences from claim 1, there was no amplification with these genera and thus Kong does not teach amplification of two targets that have less than 50% identity.

Claim 15 (in combination with claim 14, from which claim 15 depends) recites that the mixture comprises a first primer pair and a second primer pair, each of which includes a forward primer and a reverse primer and wherein the single-stranded polynucleotide sequence comprises sequences or subsequences of the first primer pair and the second primer pair. Thus, the Examiner is not correct that "[t]he claims do not require one polynucleotide sequence compris[ing] four primer sequences" as argued on page 7, last substantive sentence, in the Final Office Action because a first pair of primers and a second pair of primers results in four primers.

In view of the above arguments, Applicants respectfully request withdrawal of the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Matthew E. Hinsch
Reg. No. 47,651

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
Attachments
MEH:meh
61115267 v1